Remarks

Reconsideration of this Application is respectfully requested. Upon entry of the foregoing amendment, claims 48-75 are pending in the application, with claims 48, 52, 54, 60 and 62-75 being the independent claims. New claims 62-75 are sought to be added.

Presentation of claims 62-75 is believed to be consonant with Applicants' previous election of Group III (i.e., methods of treating a disease or condition comprising administrating an effective amount of a lectin). *See* Reply to Restriction Requirement, filed February 27, 2004; and Office Communication mailed January 29, 2004, "Supplemental Election/Restrictions," beginning on page 3. Hence, claims 54-75 are consonant with Applicants' previous election and are believed to be properly before the Examiner.[1]

Support for these new claims can be found throughout the specification and the previously presented claims. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections and that they be withdrawn.

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1 Applicants note that it was previously stated that claims 48-61 were believed to be consonant with Applicants' previous election. *See* Amendment and Reply Under 37 C.F.R. § 1.111, filed September 21, 2004, at page 6, paragraph 2. this statement, however, is incorrect. Only previously presented claims 54-61 read on Applicants' previous election. It is respectfully requested that claims 48-53 remain pending (and not withdrawn) until such time as Applicants' petition to withdraw the restriction requirement (discussed herein) is granted or denied.

I. Outstanding Petition to Director Under 37 C.F.R. §§ 1.144 and 1.181 to Withdraw Final Restriction Requirement

Applicants respectfully remind the Examiner that a petition to the director under 37 C.F.R. §§ 1.144 and 1.181 to withdraw the final restriction requirement was timely filed for the captioned application on August 17, 2004. Applicants request that the Examiner defer issuing further Office Actions for this application until the USPTO has acted upon this petition.

II. Enablement Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 48-61 are rejected under 35 U.S.C. § 112, first paragraph. In particular, the examiner has alleged that the specification "does not reasonably provide enablement for treatment of a C-fibre neuron associated diseases/conditions with any ECL conjugate, other than ECL- LH_N/A." Office Action, page 2, lines 14-16. Applicants respectfully traverse the rejection.

A. Legal Requirement for Setting forth a Prima Facie Case of Non-Enablement

Reflecting the decisions of the federal courts, the M.P.E.P. provides guidance to examiners regarding enablement rejections. *See* M.P.E.P., 8th ed., § 2164 (Rev. 2, May 2004). In particular, the M.P.E.P. states that "[i]n order to make a rejection, the examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention." *Id.* at §2164.04 (*citing In re Wright*, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993)). Moreover, any allegation doubting "the truth or accuracy of any statement in a supporting disclosure . . . [must be supported by] acceptable evidence or reasoning which is inconsistent with the contested statement." *In*

re Marzocchi, 439 F.2d 220, 224, 169 USPQ 367 (CCPA 1971). As with the written description requirement, an enablement analysis must be performed from the perspective of the skilled artisan: "The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation." *United States v. Telectronics, Inc.*, 857 F.2d 778, 785, 8 USPQ2d 1217, 1223 (Fed. cir. 1988).

Moreover, the Federal Circuit has stated that "[t]he specification need not disclose what is well known in the art." *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991) (*see also Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986) ("a patent need not teach, and preferably omits, what is well known in the art."), *cert. denied*, 480 U.S. 947 (1987); *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984); *In re Myers*, 410 F.2d 420, 424 (C.C.P.A. 1969) ("A specification is directed to those skilled in the art and need not teach or point out in detail that which is well-known in the art.").

B. The Specification Enables the Claims for the Skilled Artisan

Although Applicants argue in section ***II.C.*** below that the Examiner has not set forth a *prima facie* case of non-enablement, this section is provided to direct the Examiner's attention to exemplary portions of the specification that enable the claims for the skilled artisan. This section also refers the Examiner to additional objective evidence which demonstrates that the claims are enabled.

1. The Claimed Methods are Enabled as to Any Conjugate-Free Galactosyl- or Glucosyl-Binding Lectin

Applicants respectfully stress that it is the **lectin component** of the present invention that is the biologically active molecule. The efficacy of this molecule has been demonstrated in the present specification for representative galactosyl-binding lectins ECL and IB4. *See, e.g.,* Specification, Examples 8, 14, 16, 18 and 19. The efficacy of this molecule has also been demonstrated in the present specification for a representative glucosyl-binding lectin, WGA. *See, e.g.,* Specification, Example 7. It is the lectin component of the compositions used in the claimed methods which target specific sugar residues, which are characteristic markers present on the cell surface of C-fibres. *See* three abstracts provided herewith as Exhibit 1. Thus, the efficacy of the compositions used in the claimed methods (either lectin alone or conjugated) stems from the lectin component of the composition.

Prior to the present invention, it would have been routine for a skilled person to select or obtain a galactosyl- or glucosyl-binding lectin as recited in the pending claims. This is abundantly clear from the present specification, which confirms that such lectins were commercially available. *See, e.g.,* Specification, page 7, lines 9-13. Prior to the present application, such lectins could also be routinely extracted and purified from nature. *See, e.g.,* Specification, page 9, lines 7-9.

Hence, Applicants' specification clearly teaches the skilled artisan that galactosyl- or glucosyl-binding lectins can be used alone (i.e., absent any other conjugate) according to the newly claimed methods. Moreover, Applicants' specification clearly indicates that such lectins were either commercially available or could otherwise routinely be isolated.

Thus, Applicants' specification fully enables the use of lectins (free of any conjugate) as the composition of the claimed methods.

2. The Claimed Methods are Enabled as to Any Endopeptidase-Free Conjugate of a Galactosyl- or Glucosyl-Binding Lectin

Consistent with the above comments, efficacy of the *conjugate* aspect of the present invention also stems from the lectin component. Thus, selection of an accompanying peptide or protein for use in a conjugate of the present invention would be routine to a skilled person as the peptide or protein need not provide any technical effect. In the context of the present invention, the peptide or protein component may be simply considered an inert "carrier" molecule. *See, e.g.,* Specification, page 10, lines 5-8. This has been confirmed by data presented in the present specification showing that a conjugate comprising lectin and an inert carrier molecule (*e.g.,* an endopeptidase-negative LH_N molecule) has efficacy in accordance with the present invention. *See, e.g.,* Specification, Example 17.

As is self-evident from the above, the only requirement of the peptide or protein component is that it lacks endopeptidase activity. In this regard, it would have been routine (prior to the present invention) for a skilled person to confirm that any such peptide or protein lacks endopeptidase activity. As objective evidence of this assertion, Applicants respectfully refer the Examiner to documents AS21 and AT21 cited in the Second Supplemental Infromation Disclosure Statement, filed in the captioned application on December 9, 2004. These documents are declarations filed under 37 C.F.R. § 1.132 submitted in Applicants' copending application 09/529,130.

Moreover, the skilled artisan would be able to make conjugates of galactosyl- or glucosyl-binding lectins suitable for use in the present invention. Basic conjugation and coupling reactions are illustrated in Applicants' specification, for example, Examples 3, 4, 9, 10, and 11. These examples illustrate conjugation and coupling of lectins that are representative of the two classes of lectin relevant to the present invention to a separate peptide or protein component. Any conventional conjugation or coupling chemistry may be employed to prepare a conjugate for use according to the claims. Applicants refer the Examiner to the aforementioned documents AS21 and AT21 as additional objective evidence regarding this assertion. Applicants also refer the Examiner to U.S. Patent No. 5,433,946 ("the '946 patent"), previously submitted as document AA1 in an Information Disclosure Statement filed on September 26, 2001. The Abstract of the '946 patent provides further objective evidence that the basic concept of coupling lectins to proteins or peptides would be considered routine by the skilled artisan.

Hence, Applicants' specification clearly teaches the skilled artisan that endopeptidase-free conjugates of galactosyl- or glucosyl-binding lectins can be used according to the newly claimed methods. Applicants' specification also clearly indicates that such conjugates can routinely be made. Moreover, Applicants' have provided additional objective evidence that such conjugates can routinely be made. Thus, Applicants' specification fully enables the use of endopeptidase-free galactosyl- or glucosyl-binding lectins as the composition of the claimed methods.

3. The Claimed Methods are Enabled as to Administration of Either Conjugate-Free or Conjugated Galactosyl or Glucosyl Binding Lectins

Applicants' specification describes formulations and modes for administering either conjugate-free or conjugated galactosyl- or glucosyl-binding lectins according to the claimed methods. *See, e.g.,* Specification, pages 13-14. Particularly preferred administrations are described, for example, Examples 5, 6, 7, 8, 16, 18 and 19 of the specification. Hence, the claimed methods are enabled as to administration of either conjugate-free or conjugated galactosyl- or glucosyl-binding lectins.

C. A Prima Facie Case for a Non-Enablement Rejection Has Not Been Set Forth

1. The Skilled Artisan is Adequately Guided by Applicants' Specification

The Examiner has alleged that

> Enablement must be provided by the specification unless it is well known in the art. . . . A search of the prior art, as to ECL conjugates for treating C-fiber related disorders, revealed a very limited number of teachings directed to the specific invention of the present application, therefore, the use of ECL conjugates for treating C-fiber related disorders cannot be construed as being well known in the art, and thus reliance for enablement must stem from the specification.

Office Action, page 3, last paragraph. However, this statement misconstrues the level of guidance needed for the skilled artisan. Indeed, *in every novel and unobvious invention there will be a very limited number of teachings directed to the specific invention.* If such teachings existed, the invention would lack novelty or be obvious. It is important to note that the claimed subject matter of the captioned application is free of the prior art.

The relevant question is whether the skilled person could reasonably make and use lectins or conjugates thereof that possess the requisite properties, and thus reproduce

the claimed method(s). Here, Applicants' specification clearly identifies galactosyl and glucosyl binding lectins as having a biological effect useful for the claimed methods. Guided by this new and unobvious teaching, the skilled artisan will readily understand that such lectins can be used in an unconjugated or conjugated form. Where they are used in a conjugated form, the skilled artisan can couple the lectin to a peptide or protein using any number of well known and routine coupling techniques. *See* documents AS21 and AT21 cited in the Second Supplemental Infromation Disclosure Statement, filed in the captioned application on December 9, 2004.

The Examiner has not provided *any* evidence why the skilled artisan could only practice Applicants' invention with undue experimentation. Such evidence should begin with a description of the skilled artisan and his or her knowledge to provide the proper perspective and framework from which to evaluate whether Applicants' claims are enabled. However, the Examiner entirely fails to define the skilled artisan or the knowledge generally known to him or her at the time the application was filed. Hence, the Examiner has not set forth a *prima facie* case that the claims are not enabled by the specification.

2. Scope of Lectin or Lectin Conjugate

The Examiner has also alleged that, because "[t]here are no working examples to indicate whether other ECL conjugates would be enabled (and what the structure/function of these are)," the skilled artisan would only be able to practice the claimed invention through undue experimentation. Office Action, page 4, first full

paragraph. The Examiner's statement appears to be directed to both the scope of lectin and the scope of the peptide or protein used in a conjugate.

Regarding selection of the lectin component of the present invention, the pending claims are concerned with only two well-characterized classes of lectin.[2] Thus, there can be no undue burden in selecting and using such molecules. Moreover, Applicants have demonstrated in the present specification that these molecules modulate C-fibre activity. In this regard, the only relevant consideration in the context of the present invention is their ability to bind glucose or galactose residues on C-fibres. By definition, all galactosyl-binding lectins bind to galactosyl residues and all glucosyl-binding lectins bind to glucose residues. Hence, any galactosyl- or glucosyl-binding lectin would be suitable for use in the present invention. Moreover, the Examiner has not provided *any* evidence (or even reasoning) to support the allegation that selection of a lectin is unduly burdensome for the skilled artisan.

Regarding selection of the peptide or protein component of the present invention, as discussed above, this molecule is not relevant to efficacy considerations. Thus, consistent with lines 14-15 on page 10 of the specification, a skilled person would consider any peptide or protein to be suitable for use in the present invention, so long as the peptide or protein is free from endopeptidase activity, which is a readily testable property. Again, Applicants have demonstrated efficacy for one such inert peptide or protein molecule. Moreover, the Examiner has not provided any evidence (or even reasoning) to support his lack of enablement assertion. Hence, the skilled artisan would

not face undue experimentation in order to select a peptide or protein with which to make a lectin conjugate.

3. Scope of LH_N Serotype

Applicants note that the Examiner appears to question the scope of LH_N. In particular, in stating that the specification "does not reasonably provide enablement for treatment of a C-fibre neuron associated diseases/conditions with any ECL conjugate, other than ECL- LH_N/A," the Examiner appears to be stating that a galactosyl- or glucosyl-binding lectin conjugated to LH_N is only enabled where the LH_N is derived from botulinum neurotoxin serotype A. To the extent the Examiner intended to make such an enablement scope rejection, Applicants traverse the rejection.

Such a rejection is unreasonable for at least three reasons. First, as mentioned above, the peptide or protein component of a conjugate of the present invention is immaterial to efficacy. Secondly, Clostridial neurotoxins have been well-known for many years, and collectively form a class of very closely, structurally related molecules. *See* Exhibit 2, provided herewith. Thus, having demonstrated efficacy of a conjugate comprising an inert serotype A component (see Example 17 of the present specification), a skilled person would expect efficacy with conjugates comprising corresponding inert peptide or protein components from other Clostridial serotypes. Thirdly, the Examiner has provided no support for his assertion that, despite clear evidence in the present

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2 Lectins are routinely classified according to the sugar residues to which they bind. *See* documents AR15 and AT15 cited in Applicants' Second Supplemental IDS, filed December 9, 2004.

specification in favor conjugates comprising inert serotype A components, conjugates containing corresponding inert non-serotype A components would not have efficacy.

D. Summary of Enablement Rejection

The Examiner may properly withdraw the enablement rejection of claims 48-61 because Applicants' specification adequately enables the skilled artisan as to how to make and use galactosyl- or glucosyl-binding lectins or conjugates thereof according to the claimed methods. Moreover, the rejection may properly be withdrawn because a *prima facie* case in support of the rejection has not been set forth. Accordingly, Applicants request that the Examiner reconsider and withdraw the enablement rejection of claims 48-61.

III. Indefiniteness Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 48-61 are rejected under 35 U.S.C. 112, second paragraph. In particular, the Examiner alleges that these claims are "indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." Office Action, page 4. In particular, the Examiner alleges that

> Applicant was required to elect a single compound, and identifying its chemical structure, as the invention (Supplemental Restriction). This requirement still has not been met (i.e. a specific lectin, for instance, the ECL conjugate ECL-LH_N/A) and the claims thus remain indefinite as to what constitutes the elected invention. As drafted, the claimed invention is essentially unsearchable (other than the elected group) because the instant claims do not contain a distinguishable structure (i.e. ECL-LH_N/A) that may be searched.

Office Action, pages 4-5. Applicants traverse the rejection.

Applicants assert that claims 48-61 are not indefinite. Independent claims 48 and 52 are genus method claims directed to the use of galactosyl- or glucosyl-binding lectins. Independent claims 54 and 60 are genus claims directed to the use of conjugates of galactosyl- or glucosyl-binding lectins. Since the method of using these lectins (or conjugates thereof) is free of the prior art, such claims possess unity of invention and should not be subject to a restriction requirement.

The Examiner has previously indicated that

> The inventions do not contain a distinguishable structure (i.e. lectin, peptide, protein, nucleic acid, conjugate, or composition) that may be searched. Therefore, as part of electing one of . . . [the restricted groups], Applicant is required to elect a specific lectin, peptide, protein, nucleic acid, conjugate, or composition . . . so that a search of the invention may be undertaken. . . . This requirement is not to be taken as an election of species, but rather as an election of a single invention . . . .

Office Action mailed January 29, 2004, page 8, last paragraph. *See also* Office Action mailed October 3, 2003, paragraph spanning pages 4-5.

Applicants note that all of the claims contain the distinguishable structure of a galactosyl- or glucosyl-binding lectin. Such lectins are properly grouped together because they possess a common structure and function. Because such a structure and function is common to all of the claims, the claims should be construed as method claims directed to a novel and unobvious use of a Markush group. Moreover, Applicants have already provided the Examiner with a species as a basis on which to perform the search. *See* Reply to Restriction Requirement, filed February 27, 2004. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. § 112, 2nd paragraph be withdrawn.

In the alternative, Applicants respectfully remind the Examiner that a Petition to the Director Under 37 C.F.R. §§ 1.144 and 1.181 to Withdraw Final Restriction Requirement was filed on August 17, 2004. Accordingly, if the rejection is not withdrawn, Applicants request that this rejection be held in abeyance until such time as the petition has been granted or denied.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Evidence for (galactose)-binding lectins binding to C-fibres

Notes

- ***IB4 binds to terminal galactose, is exemplified in the patent as analgesic, and continues to be the most exemplified lectin-binding event in the literature.***

ELSEVIER
FULL-TEXT ARTICLE

Tetrodotoxin-sensitive and -resistant Na+ channel currents in subsets of small sensory neurons of rats.

Wu ZZ, Pan HL. Brain Res. 2004 Dec 17;1029(2):251-8.

Department of Anesthesiology, The Pennsylvania State University College of Medicine, 500 University Drive, The Milton S. Hershey Medical Center, Hershey, PA 17033-0850, USA.

Voltage-activated Na+ channels in the primary sensory neurons are important for generation of action potentials and regulation of neurotransmitter release. The Na+ channels expressed in different types of dorsal root ganglion (DRG) neurons are not fully known. In this study, we determined the possible difference in tetrodotoxin-sensitive (TTX-S) and -resistant (TTX-R) Na+ channel currents between isolectin B4 (IB4)-positive and IB4-negative small DRG neurons. Whole-cell voltage- and current-clamp recordings were performed in acutely isolated DRG neurons labeled with and without IB4 conjugated to Alexa Fluor 594. The peak Na+ current density was significantly higher in IB4-negative than IB4-positive DRG neurons. While all the IB4-negative neurons had a prominent TTX-S Na+ current, the TTX-R Na+ current was present in most IB4-positive cells. Additionally, the evoked action potential had a higher activation threshold and a longer duration in IB4-positive than IB4-negative neurons. TTX had no effect on the evoked action potential in IB4-positive neurons, but it inhibited the action potential generation in about 50% IB4-negative neurons. This study provides complementary new information that there is a distinct difference in the expression level of TTX-S and TTX-R Na+ channels between IB4-negative than IB4-positive small-diameter DRG neurons. This difference in the density of TTX-R Na+ channels is responsible for the distinct membrane properties of these two types of nociceptive neurons.

PMID: 15542080 [PubMed - indexed for MEDLINE]

ELSEVIER
FULL-TEXT ARTICLE

The behavioral and neuroanatomical effects of IB4-saporin treatment in rat models of nociceptive and neuropathic pain.

Tarpley JW, Kohler MG, Martin WJ. Brain Res. 2004 Dec 10;1029(1):65-76.

Department of Pharmacology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA.

One distinguishing feature of primary afferent neurons is their ability to bind the lectin IB(4). Previous work suggested that neurons in the inner part of lamina II (IIi), onto which IB(4)-positive sensory neurons project, facilitate nociceptive transmission following tissue or nerve injury. Using an IB(4)-saporin conjugate (IB(4)-SAP), we examined the contribution of IB(4)-positive neurons to nociceptive processing in rats with and without nerve injury. Intrasciatic injection of IB(4)-SAP (5 mug/5 mul) significantly decreased IB(4)-labeling and immunoreactive P(2)X(3) in the spinal cord and delayed the behavioral and neuroanatomical consequences of L5 spinal nerve ligation (SNL) injury. In the absence of injury, thermal and mechanical nociceptive thresholds increased 2 weeks post-treatment only in IB(4)-SAP-treated, but not control (saline or saporin only), rats. Acute NGF-induced hyperalgesia was also attenuated following IB(4)-SAP treatment. In the SNL model, mechanical allodynia failed to develop 1 and 2 weeks post-injury, but was fully established by 4 weeks. Moreover, neuropeptide Y immunoreactivity (NPY-ir), which increases in the spinal cord after nerve

injury, was unchanged in IB(4)-SAP-treated animals whereas immunoreactive PKCgamma decreased 2, but not 4, weeks post-injury. Quantitative RT-PCR revealed a reduction in P(2)X(3) mRNA in L4 DRG of IB(4)-SAP-treated animals, but no change in TrkA expression. Our results suggest that IB(4)-positive neurons in L4 are required for the full expression of NGF-induced hyperalgesia and participate in the behavioral and anatomical consequences that follow injury to the L5 spinal nerve.

PMID: 15533317 [PubMed - indexed for MEDLINE]

Analysis of the distribution of binding sites for the plant lectin Bandeiraea simplicifolia I-isolectin B4 on primary sensory neurones in seven mammalian species.

Gerke MB, Plenderleith MB. Anat **Rec. 2002 Oct 1;268(2):105-14.**

Neuroscience Laboratory, School of Life Sciences, Queensland University of Technology, Brisbane, Queensland 4001, Australia.

The purpose of the present study was to investigate the binding patterns of the plant lectin Bandeiraea simplicifolia I-isolectin B(4) (BSI-B(4)) to sensory neurones in seven mammalian species. The dorsal root ganglia and spinal cords of three rats, mice, guinea pigs, rabbits, flying foxes, cats, and marmoset monkeys were screened for BSI-B(4) using lectin histochemistry. BSI-B(4) binding was associated with the soma of predominantly small-diameter primary sensory neurones in the dorsal root ganglia and their axon terminals within laminae I and II of the superficial dorsal horn in all seven species. The similarities of lectin binding patterns in each of these species suggest that the glycoconjugate to which BSI-B(4) binds has a ubiquitous distribution in mammals, and supports the proposal that this lectin may preferentially bind to a subpopulation of sensory neurones with a similar functional róle in each of these species.

PMID: 12221716 [PubMed - indexed for MEDLINE]

H-chains have two distinct functions, namely binding (ie. to a target cell), and translocation (ie. across an endosomal membrane). The carboxy-terminal portion (H_C) of a H-chain is involved in the high affinity, neurospecific binding of the toxin to cell surface receptors, whereas the amino-terminal portion (H_N) of the H-chain is central to the translocation of the toxin into the neuronal cell. These two functions have been extensively studied and characterised, and have been mapped to distinct portions within the H-chain [see, for example, Kurazono et al (1992) J. Biol. Chem. 267, 21, pp.14721-14729; Poulain et al (1989) Eur. J. Biochem. 185, pp. 197-203; Zhou et al (1995), Biochemistry, 34, pp. 15175-15181; Blaustein et al (1987) FEBS Letts., 226, No. 1, pp. 115-120].

L-chains possess a protease function (zinc-dependent endopeptidase activity) and exhibit a high substrate specificity for vesicle and/or plasma membrane associated proteins involved in the exocytic process. L-chains from different clostridial species or serotypes may hydrolyse different but specific peptide bonds in one of three substrate proteins, namely synaptobrevin, syntaxin or SNAP-25. These substrates are important components of the neurosecretory machinery.

By way of specific example, for botulinum neurotoxin serotype A, the above functions have been mapped to amino acid residues 872-1296 for the H_C portion, amino acid residues 449-871 for the H_N portion, and residues 1-448 for the L-chain [see Lacy, D.B. & Stevens, R.C. (1999). Sequence homology and structural analysis of the clostridial neurotoxins. J. Mol. Biol. 291, 1091-1104].

All three of the above-identified domains (ie. H_C, H_N, and L) are necessary for the *in vivo* activity of a native neurotoxin, which neurotoxin may cause prolonged muscular paralysis in an affected individual. Corresponding binding, translocation, and protease functions are necessary for the *in vivo* activity of other non-cytotoxic, bacterial toxins.

It has been well documented in the art that toxin molecules may be re-targeted to a cell that is not the toxin's natural target cell. When so re-targeted, a toxin is capable of binding to a desired target cell and, following subsequent translocation into the cytosol, is capable of exerting its effect on the target cell.

For example, in the context of non-cytotoxic toxin molecules, it has been well documented that a clostridial neurotoxin may be re-targeted by incorporation of a Targeting Moiety (TM), which is not the natural TM of a clostridial neurotoxin. The described chemical conjugation and recombinant methodologies are now regarded as conventional.

In more detail, the following patent publications, in the name of the present Applicant, describe the preparation of modified bacterial conjugates.

WO94/21300 describes the preparation of modified clostridial neurotoxin molecules that, once translocated into the cytosol of a desired target cell, are capable of regulating Integral Membrane Protein (IMP) density present at the cell surface of the target cell. The modified neurotoxin molecules are thus capable of controlling cell activity (eg. glucose uptake) of the target cell.

WO96/33273 describes the preparation of modified clostridial neurotoxin molecules that target peripheral sensory afferents. Once delivered into the cytosol of a peripheral sensory afferent, the modified neurotoxin molecules are capable of demonstrating an analgesic effect.

WO98/07864 describes the preparation of single chain, modified clostridial neurotoxin molecules, which single chain molecules are substantially inactive in terms of sequential binding, translocation and L-chain dependent endopeptidase activities. The single chain molecules are activatable into active di-chain molecules through a proteolytic cleavage reaction.

WO99/17806 describes the preparation of modified clostridial neurotoxin molecules that target primary sensory afferents, which modified neurotoxins are capable of demonstrating an analgesic effect.

WO00/10598 describes the preparation of modified clostridial neurotoxin molecules that target mucus hypersecreting cells (or neuronal cells controlling said mucus hypersecreting cells), which modified neurotoxins are capable of inhibiting hypersecretion from said cells.

WO01/21213 describes the preparation of modified clostridial neurotoxin molecules that target a wide range of different types of non-neuronal target cells. When so targeted and delivered into the cytosol, the modified molecules are capable of preventing secretion from the target cells.

Additional publications in the technical field of re-targeted toxin molecules include:- WO00/62814; WO00/04926; US5,773,586; WO93/15766; WO00/61192; WO99/58571; and US2003/0059912.